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EVALUATION OF TRIACID AND DRY ASHING PROCEDURES FOR DETERMINING POTASSIUM, CALCIUM, MAGNESIUM, IRON, ZINC, MANGANESE, AND COPPER IN PLANT MATERIALS

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ABSTRACT

Dry ashing and wet ashing techniques are routinely used for preparing plant materials for elemental analysis. The two techniques generally give similar results for the analysis of plant materials but differences have been observed in results using the two methods for elements such as calcium (Ca), iron (Fe), and zinc (Zn). We made a study to compare the dry ashing and triacid digestion procedures for determining potassium (K), Ca, magnesium (Mg), Fe, manganese (Mn), copper (Cu), and Zn in sorghum and rice plant samples having a range in concentration of these elements. For sorghum, the two procedures gave similar results for the determination of K,

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Mg, Mn, and Zn, the dry ashing procedure gave higher values for Ca, Fe, and Cu than those obtained using the triacid digestion procedure. For rice, the two procedures gave similar results for all nutrient elements except Ca for which the values were higher by the dry ashing procedure than with the triacid digestion procedure. Our results suggest that dry ashing technique provides more reliable results for the analysis of sorghum and rice plant materials for Ca and may be preferred over the triacid digestion procedure. Also, for sorghum plant samples dry ashing seemed more reliable for determination of Fe and Cu. Both dry ashing and triacid digestion methods appeared satisfactory for the determination of K, Mg, Mn, and Zn.

INTRODUCTION

In preparing plant materials for elemental analysis, organic matter in plant tissue is destroyed using combustion at high temperature. This process is termed dry ashing or wet ashing when acid mixtures are used to digest the materials (1,2). These two techniques of preparing plant materials for elemental analysis have generated a lot of interest as well as controversy.

It is generally accepted that both techniques can give comparable results for most elements, although exceptions exist (3). For example, boron (B) may be lost through volatilization during wet ashing and, consequently, lower results have been reported using wet ashing as compared to dry ashing. Differences in the results using the two techniques have been reported for elements such as aluminum (Al), iron (Fe), zinc (Zn), and calcium (Ca).

For routine analysis of plant materials for various elements, it is important results are compared using dry ashing and wet ashing techniques. While wet ashing technique based on digestion with triacid may be rapid relative to dry ashing, the real choice of the technique must be based on comparative evaluation of the two techniques with a range of materials differing in elemental concentrations of nutrients. It has been suggested that the method of organic matter destruction is dictated by the elements to be determined as well as by the elemental content of the plant tissue (3). With this objective, wet ashing with triacid digestion and dry ashing techniques were evaluated for determining potassium (K), calcium (Ca), magnesium (Mg), Fe, Zn, manganese (Mn), and copper (Cu) in sorghum [*Sorghum* *bicolor* (L.) Moench] and rice (*Oryza sativa* L.) plant samples known to vary in concentration of various elements.

MATERIALS AND METHODS

Plant Materials

Sorghum plant samples comprising of stalk and grain samples at harvest, selected to have a range in nutrient elements, were used in the study to compare triacid and dry ashing procedures. The plant materials were finely ground (<40 mesh for stalk and <60 mesh for grain samples) then oven dried at 60°C for 48 h before analysis by wet and dry ashing. Rice plant samples from upland and lowland ecologies were selected to obtain a range in concentrations of various elements and included to provide further evaluation of the techniques for analyzing plant samples of another plant species.

Methods

Triacid Digestion

Ground and dried plant materials weighing 0.5 g were transferred to 125 mL conical digestion flasks. Twelve (12) mL of triacid mixture of nitric acid, sulfuric acid and perchloric acid (9:2:1 (v/v)) were added to the flasks. Plant materials were digested in cold for 3 h followed by digestion for 2–3 h on a hot plate, until the digest was clear or colorless. The flasks were allowed to cool and the contents were diluted to an appropriate volume.

Dry Ashing

Ground and dried plant materials, 0.5 g, were weighed into 30 mL silica crucibles. The crucibles were placed in a muffle furnace at room temperature. The furnace temperature was set at 470°C, and at the temperature was gradually raised. The plant samples were ashed for 16 h by leaving them overnight. Cool crucibles were taken out and the ash was moistened with a few drops of water, followed by 3 mL of 5 *M* hydrochloric acid. The contents were heated on a hot plate at about 80°C to dissolve the ash.

Potassium, Ca, Mg, Mn, Zn, Fe, and Cu in the digests were determined using atomic absorption spectrophotometry.

RESULTS

Analysis of Sorghum Plant Samples

The results on the analysis of sorghum plant samples for K, Ca, Mg, Fe, Zn, Mn, and Cu using triacid and dry ashing techniques (Tables 1 and 2) showed that there was a good agreement between the two digestion procedures results for K, Mg, Mn, and Zn. But there was a poor agreement between the two digestion

Table 1. Comparison of Values of K, Ca, and Mg in Sorghum Plant Samples Determined by Triacid Digestion (TA) and Dry Ashing (DA) Procedures

	K (%)		Ca (%)		Mg (%)	
Sample No.	TA	DA	TA	DA	TA	DA
1	1.25	1.31	0.24	0.29	0.14	0.16
2	2.19	2.26	0.24	0.31	0.14	0.15
3	2.07	2.00	0.24	0.31	0.17	0.18
4	2.12	2.20	0.22	0.37	0.16	0.17
5	1.12	1.14	0.21	0.24	0.16	0.17
6	1.21	1.35	0.29	0.34	0.17	0.19
7	1.21	1.27	0.27	0.29	0.14	0.17
8	1.17	1.24	0.25	0.26	0.14	0.17
9	1.16	1.27	0.24	0.32	0.14	0.15
10	1.64	1.60	0.21	0.33	0.14	0.14
11	1.78	1.86	0.18	0.30	0.14	0.14
12	2.19	2.18	0.25	0.29	0.14	0.15
13	2.45	2.63	0.22	0.32	0.11	0.12
14	2.13	2.15	0.21	0.32	0.14	0.16
15	2.23	2.41	0.29	0.33	0.16	0.17
16	2.38	2.32	0.28	0.31	0.16	0.17
17	2.02	1.95	0.26	0.29	0.13	0.12
18	1.95	1.92	0.25	0.29	0.17	0.18
19	1.99	2.12	0.18	0.26	0.14	0.15
20	1.85	1.91	0.26	0.43	0.15	0.17
21	2.22	2.09	0.26	0.31	0.19	0.19
22	1.75	1.81	0.18	0.32	0.17	0.23
23	2.18	2.39	0.22	0.39	0.17	0.19
24	1.81	1.96	0.21	0.38	0.17	0.19
25	2.44	2.63	0.21	0.41	0.14	0.15
26	2.15	2.24	0.25	0.35	0.14	0.16
27					0.18	0.19

Table 2. Comparison of Values of Fe, Zn, Mn, and Cu in Sorghum Plant Samples Determined by Triacid Digestion (TA) and Dry Ashing (DA) Procedures

	Fe ($mg\,kg^{-1}$)		Zn ($mg\,kg^{-1}$)		Mn ($mg\,kg^{-1}$)		Cu ($mg\,kg^{-1}$)	
Sample No.	TA	DA	TA	DA	TA	DA	TA	DA
1	115	150	69	68	26	25	10	8
2	108	146	53	54	28	29	8	5
3	126	155	43	47	34	31	7	9
4	109	163	30	34	35	37	7	16
5	127	157	80	80	28	24	10	9
6	122	120	69	72	26	27	10	4
7	100	124	63	66	24	22	9	11
8	99	192	74	87	25	24	9	8
9	121	140	77	68	29	25	16	10
10	119	168	49	38	27	27	9	4
11	141	237	50	55	26	28	7	4
12	113	151	52	53	25	24	7	4
13	97	101	54	55	29	27	9	6
14	103	130	58	53	32	36	7	4
15	105	117	58	61	28	29	7	6
16	87	140	48	47	27	28	7	9
17	109	127	47	52	35	33	5	9
18	97	140	52	57	31	28	7	8
19	94	99	38	41	29	28	7	15
20	243	203	31	35	31	31	9	6
21	114	141	41	51	38	37	9	10
22	97	121	28	31	32	35	7	6
23	148	195	34	34	38	39	7	9
24	94	194	33	38	40	45		
25	118	164	30	37	26	30		
26			25	30	38	38		

methods for Ca, Fe, and Cu and the concentrations of Ca, Fe, and Cu determined by the dry ashing procedure were higher than those obtained by the triacid digestion procedure.

There was an excellent agreement ($R^2 = 0.958$, $n = 26$) in the values of K in plant samples determined by triacid and dry ashing procedures. Good to excellent agreement was also obtained between the two digestion procedures for Mg ($R^2 = 0.712$, $n = 27$), Mn ($R^2 = 0.825$, $n = 26$) and Zn ($R^2 = 0.900$, $n = 26$). But there was a poor agreement between the two digestion methods for the determination of Fe ($R^2 = 0.283$, n = 25) Ca ($R^2 = 0.006$, n = 26) and Cu ($R^2 = 0.004$, n = 23) (Table 3). The concentrations of Ca, Fe, and Cu in plant materials, determined by the dry ashing method, were higher than those obtained by the triacid digestion procedure (Tables 1 and 2).

Comparison of the triacid and dry ashing procedures for determining K, Ca, Mg, Fe, Zn, Mn, and Cu showed that the two techniques generally had similar standard deviation (SD) for the various elements (Table 4).

Analysis of Rice Plant Samples

To provide additional evidence on the efficacy of the triacid and dry ashing techniques for analysis of plant materials, rice plant samples having a range in K, Ca, Mg, Fe, and Zn concentrations were analyzed using the two methods. Results with rice plant samples showed that except for Ca, both triacid digestion and dry ashing methods gave similar results (Table 5). For Ca determination, the dry ashing procedure provided higher values than those obtained with triacid digestion method. The SD for the two methods for various elements were comparable.

Our results thus provide evidence which show that for determination of Fe in rice plant materials, the results were comparable for the two procedures. While in the case of sorghum plant samples, dry ashing procedure provided more reliable results than triacid digestion method.

DISCUSSION

For both sorghum and rice plant materials, the dry ashing procedure provided a more reliable determination of Ca than triacid digestion. The lower

Table 3. Relationship Between the Values of K, Ca, Mg, Fe, Zn, Mn, and Cu in Sorghum Plant Samples Determined by Triacid (TA) and Dry Ashing (DA) Procedures

Element	Regression Equation	R^2
K	DA–K = 0.06 + 1.00 TA–K	0.958 (n = 26)
Ca	DA–Ca = 0.31 + 0.035 TA–Ca	0.006 (n = 26)
Mg	DA–Mg = −2.11 + 1.07 TA–Mg	0.712 (n = 27)
Fe	DA–Fe = 82.88 + 0.586 TA–Fe	0.283 (n = 25)
Zn	DA–Zn = 6.50 + 0.91 TA–Zn	0.900 (n = 26)
Mn	DA–Mn = −3.03 + 1.10 TA–Mn	0.825 (n = 26)
Cu	DA–Cu = 7.58 + 0.03 TA–Cu	0.004 (n = 23)

Table 4. Comparison of Triacid Digestion and Dry Ashing Methods for Determination of K, Ca, Mg, Fe, Zn, Mn, and Cu in Sorghum Plant Samples

		Triacid Method			Dry Ashing Method		
Element	No. of Samples	Range	Mean	SD	Range	Mean	SD
K ($g\,100\,g^{-1}$)	26	1.12–2.45	1.87	0.432	1.14–2.63	1.93	0.441
Ca ($g\,100\,g^{-1}$)	26	0.18–0.29	0.24	0.032	0.24–0.43	0.32	0.045
Mg ($g\,100\,g^{-1}$)	27	0.11–0.19	0.15	0.018	0.12–0.23	0.16	0.024
Fe ($mg\,kg^{-1}$)	25	87–243	116	30.3	99–237	151	33.3
Zn ($mg\,kg^{-1}$)	26	25–80	49	15.9	30–87	51	15.4
Mn ($mg\,kg^{-1}$)	26	24–40	30	4.7	22–45	30	5.7
Cu ($mg\,kg^{-1}$)	23	5–16	8	2.1	4–16	8	3.3

Table 5. Comparison of Triacid Digestion and Dry Ashing Methods for Determination of K, Ca, Mg, Fe, and Zn in Rice Plant Samples

		Triacid Method			Dry Ashing Method		
Element	No. of Samples	Range	Mean	SD	Range	Mean	SD
K ($g\,100\,g^{-1}$)	17	1.44–3.25	2.49	0.51	1.46–3.19	2.48	0.51
Ca ($g\,100\,g^{-1}$)	17	0.17–0.34	0.26	0.05	0.24–0.39	0.31	0.04
Mg ($g\,100\,g^{-1}$)	17	0.18–0.37	0.26	0.06	0.17–0.36	0.27	0.06
Fe ($mg\,kg^{-1}$)	17	109–719	329	168	153–726	338	165
Zn ($mg\,kg^{-1}$)	17	24–55	37	9	26–53	37	9

recovery of Ca by triacid and diacid mixtures in which sulfuric acid is used, has been attributed to the formation of sparingly soluble calcium sulfate. However, we have observed that the recovery of Ca can be improved by leaving the diluted digests overnight. In the diluted digests, a complete dissolution of calcium sulfate formed during the digestion of the plant materials takes about 16 h and hence keeping the diluted digests overnight improves the recovery of Ca.

Differences, however, were observed between the two methods of plant analysis for determining Fe. While for sorghum plant samples, dry ashing gave higher values than the triacid method for Fe, the results were closer for the two methods in the case of rice plant materials. In general, Fe concentration in rice plant samples were much higher than in the sorghum plant tissue. This is caused by increased availability of Fe following soil reduction and conversion of Fe(III) to Fe(II) under submerged wetland rice conditions (4,5).

These differences in the results may be due to differences in the content of Si in the two plant species. Rice plant samples had higher concentration of Si than sorghum plant materials. And, it has been reported that higher results are normally obtained for elements such as Al, Fe, and Zn with wet-ashing of plant tissue high in silica as compared to the results obtained using dry ashing (3).

In conclusion, our results with sorghum plant materials show that while both triacid and dry ashing procedures are equally effective for determining K, Mg, Mn, and Zn, the dry ashing technique may be preferred for determining Ca, Fe, and Cu in plant materials. Results with rice plant samples gave similar results for all nutrient elements tested except Ca for which dry ashing provided more reliable results.

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